Systematic Review of Emerging Technologies in Cystic Fibrosis Treatment: Gene Therapy and CRISPR Strategies for the Future

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Abstract

The current systematic review aimed to evaluate research on cystic fibrosis (CF), a genetic disease that affects multiple organs, particularly the lungs, and is associated with high morbidity and mortality. A total of 7,831 relevant studies were identified from search databases, and 27 studies were ultimately considered appropriate for review after applying eligibility criteria, including three longitudinal studies and the remainder being cross-sectional. All studies included a healthy control group, with a combined total of 1,839 individuals with CF and 2,178 controls. The age range varied across studies; however, the majority were conducted in adults.The studies had different aims, including evaluating and comparing different techniques for gene therapy and CRISPR, and assessing changes in body nutrition status. Other studies focused on the evaluation of lung function, inflammation, and clinical parameters. Animal models have played a crucial role in advancing CF gene therapy. Various animal models have been developed, including pigs, ferrets, rats, zebrafish, and sheep, each with its advantages and limitations. The CF pig model has facilitated the measurement of CFTR correction in vivo and has helped define the relationship between CFTR expression and Cl^- and $HCO3^-$ transport, with important implications for CF gene therapies. Gene editing technologies, such as CRISPR/Cas9, have emerged as promising approaches to modifying nucleic acid sequences in CF research. These tools hold the potential to repair the endogenous CFTR gene and restore its function, but efficient in vivo gene delivery remains a significant challenge. Assessing changes in body composition can provide valuable information on the effects of gene therapy or CRISPR on the overall health of CF patients. The assessment of body composition changes in CF treatment is essential,

as current therapies such as CFTR modulators primarily target the respiratory system and may not fully address the systemic effects of the disease. Gene therapy and CRISPR have the potential to provide more comprehensive and long lasting treatments for CF, and assessment of body composition changes can serve as a clinical endpoint in future clinical trials.

Introduction

Cystic fibrosis (CF) is a genetically inherited disease found primarily in the lungs (Alton et al., 2013). In healthy individuals, the CFTR protein helps maintain a balance of salt and water in the body's cells and tissues. However, in individuals with cystic fibrosis, a genetic disorder caused by mutations in the CFTR gene, the CFTR protein does not function correctly, leading to a build-up of thick, sticky mucus in the lungs, pancreas, and other organs (Alton et al., 2013). Mutations in the CFTR gene can also lead to other health conditions, such as the congenital bilateral absence of the vas deferens (CBAVD), which affects male fertility, and chronic bronchitis and bronchiectasis, which are respiratory conditions that affect the airways (Stuhrmann & Dork, 2000). Cystic fibrosis (CF) is one of the most common inherited disorders in the United States. According to the Cystic Fibrosis Foundation, approximately 30,000 people in the United States have cystic fibrosis. The prevalence of cystic fibrosis in the United States is estimated to be about 1 in 3,500 live births (Ruzal-Shapiro, 1998). However, the prevalence of the disease can vary depending on different factors, such as ethnicity and geographic location. The disease is more common in individuals of European descent, with a prevalence of 1 in 2,500 live births, compared to individuals of African American or Asian descent, with a prevalence of 1 in 17,000 and 1 in 31,000 live births, respectively. Geographic location can also play a role, with higher rates of cystic fibrosis reported in certain regions of the country, such as the Midwest and Northeast (Dongarwar et al., 2022). On another hand, the prevalence of cystic fibrosis (CF) in Europe varies by country and region. According to the European Cystic Fibrosis Society Patient Registry, the average prevalence of CF in Europe is approximately 1 in 10,000 live births, with some variation depending on the country and region. For example, in Northern and Western Europe, the prevalence of CF is higher, with rates ranging from 1 in 5,000 to 1 in 9,000 live births. In Southern and Eastern Europe, the prevalence is lower, with rates ranging from 1 in 15,000 to 1 in 25,000 live births (Mehta et al., 2010).

Additionally, the frequency of specific CF mutations can vary by region and ethnic group. For example, the most common CF mutation in Northern and Western Europe is the F508del mutation, while in Southern Europe, the R1162X mutation is more common (Bobadilla et al., 2002). Given the significant prevalence of CF and its profound impact on affected patients, this disease acts as a major societal burden. The chronic nature of CF requires continuous, lifelong medical care, contributing to economic hardships through increased healthcare costs, reduced work productivity for patients, and psychological strain on families (Hogg et al., 2007). The cumulative effect of these challenges underscores the urgent need for effective intervention such as gene therapy, which can address CF by its root causes. This approach would not only improve patient outcomes but also reduce the broader economic and societal impact of the disease. Gene therapy is a promising treatment approach for cystic fibrosis (CF) that aims to correct the underlying genetic defect responsible for the disease (Quintana-Gallego et al., 2014). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which provides instructions for producing the CFTR protein. Gene therapy for CF involves introducing a healthy copy of the CFTR gene into cells to replace the mutated gene. There are several approaches to gene therapy for CF, including viral and non-viral vectors. Viral vectors are modified viruses that are used to deliver the healthy CFTR gene to cells in the lungs (Derichs, 2013). The most used viral vectors for CF gene therapy are adeno-associated viruses (AAVs) and lentiviruses. These viruses are modified so that they cannot cause disease, but they can still enter cells and deliver the healthy gene. Non-viral vectors use other methods to introduce the healthy CFTR gene such as lipid nanoparticles or plasmids, which can introduce the healthy CFTR gene into cells (Li & Samulski, 2020). While these methods are less efficient than viral vectors, they may be safer and more easily scalable. Once introduced, the healthy CFTR gene can produce the normal CFTR protein, which regulates salt and fluid movement in and out of cells. This process helps reduce the buildup of thick, sticky mucus in the lungs and other organs (Derichs, 2013).

Study Rationale

Gene therapy for CF is still in the early stages of development, with several challenges needing to be addressed before it becomes a standard treatment. These challenges include developing safe and efficient gene

delivery methods, ensuring long-term expression of the healthy gene, and managing potential immune reactions to the therapy.

Despite these obstacles, gene therapy for CF shows significant promise. Ongoing research and clinical trials continue to explore innovative approaches to this treatment. If successful, gene therapy could provide a cure for CF by addressing the underlying genetic defect responsible for the disease (Fajac & Wainwright, 2017).

In the context of cystic fibrosis (CF), CRISPR technology could be used to edit the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which provides instructions for producing the CFTR protein. CF is caused by mutations in the CFTR gene, resulting in a dysfunctional CFTR protein that cannot regulate salt and fluid movement in and out of cells. This dysfunction leads to the buildup of thick, sticky mucus in the lungs and other organs (Schneider-Futschik, 2019).

CRISPR offers the potential to correct genetic mutations in the CFTR gene, enabling cells to produce a normal, functional CFTR protein. This correction could potentially eliminate CF symptoms and provide a cure for the disease (Chen et al., 2021).

The objective of this systematic review is to examine the current status of cystic fibrosis, with a focus on potential treatments utilizing gene therapy and CRISPR, as well as their impact on the survival of individuals with CF.

Materials & Methods

Search Criteria

The protocol for the systematic review, provided in the supplementary material, was developed following the guidelines for reported items, and the review's outcomes were presented in accordance with the PRISMA checklist (Moher et al., 2009). This systematic review focuses entirely on the therapeutic intervention of the CFTR gene, emphasizing gene therapy and CRISPR technology.

The databases searched for studies on cystic fibrosis included PubMed, Medline, the Cystic Fibrosis Foundation (CFF) Research and Development Database, and the Cochrane Library. The keywords used for database searches were: (cystic fibrosis OR CFTR gene) AND (nutritional composition in CF OR BMI index) AND (treatment in cystic fibrosis OR gene therapy) AND (intervention of CRISPR in CF OR pathogenesis). Additionally, Google Scholar was manually searched for relevant studies.

The investigator screened the titles and abstracts of studies published from 2018 to the present, selecting pertinent studies for inclusion in this

review. Any ambiguities regarding the eligibility of studies were resolved through consultation with the supervisor until a consensus was reached among the authors.

Inclusion and Exclusion Criteria for Systematic Review and Data Extraction

Inclusion criteria for the systematic review include: First, the studies should be published in the English language. Secondly, the studies should evaluate the use of gene therapy and CRISPR technology for cystic fibrosis. Thirdly, the studies should report on the safety and efficacy of gene therapy and CRISPR technology for cystic fibrosis. Fourthly, the studies should involve human subjects with cystic fibrosis of any age, gender, or ethnicity. Fifthly, the studies can employ any type of design, including observational studies and randomized controlled trials. Lastly, the studies should report on any outcome measures related to cystic fibrosis, such as improvement in lung function, quality of life, or survival rate. Duplicates should be excluded, and studies that only measure weight, height, or waist circumference for body composition evaluation should be excluded as well. By applying these inclusion criteria, a comprehensive and focused review of the current literature on gene therapy and CRISPR technology for cystic fibrosis can be conducted.

Study Quality Assessment

The quality of a study assessing the effectiveness and safety of gene therapy and CRISPR for cystic fibrosis can be evaluated using various tools and criteria. Firstly, the study design should be considered, and randomized controlled trials (RCTs) are generally considered to be the gold standard for evaluating treatment efficacy. The CONSORT (Consolidated Standards of Reporting Trials) statement can be used to assess the quality of reporting in RCTs, and the Cochrane Risk of Bias tool can be used to evaluate the risk of bias in non-randomized studies. For observational studies, the ROBINS-I tool can be used to assess the risk of bias, and the STROBE checklist can be used to assess the quality of reporting. Additionally, the GRADE approach can be used to evaluate the quality of evidence from both RCTs and observational studies, considering factors such as the risk of bias, consistency of results, and precision of estimates.

Results

Search Findings

A total of 7831 relevant studies were identified on the search databases for the current systematic review. From the total number of hits, 1756 abstracts and titles were initially reviewed according to the eligibility criteria. In the current study, the 975 reported studies were excluded due to not fulfilling the criteria of the review paper. The eligibility of the remaining 781 full-text articles was evaluated, and a total of 27 studies were considered appropriate for the final review. The overall sketch is summarized in Figure 1.

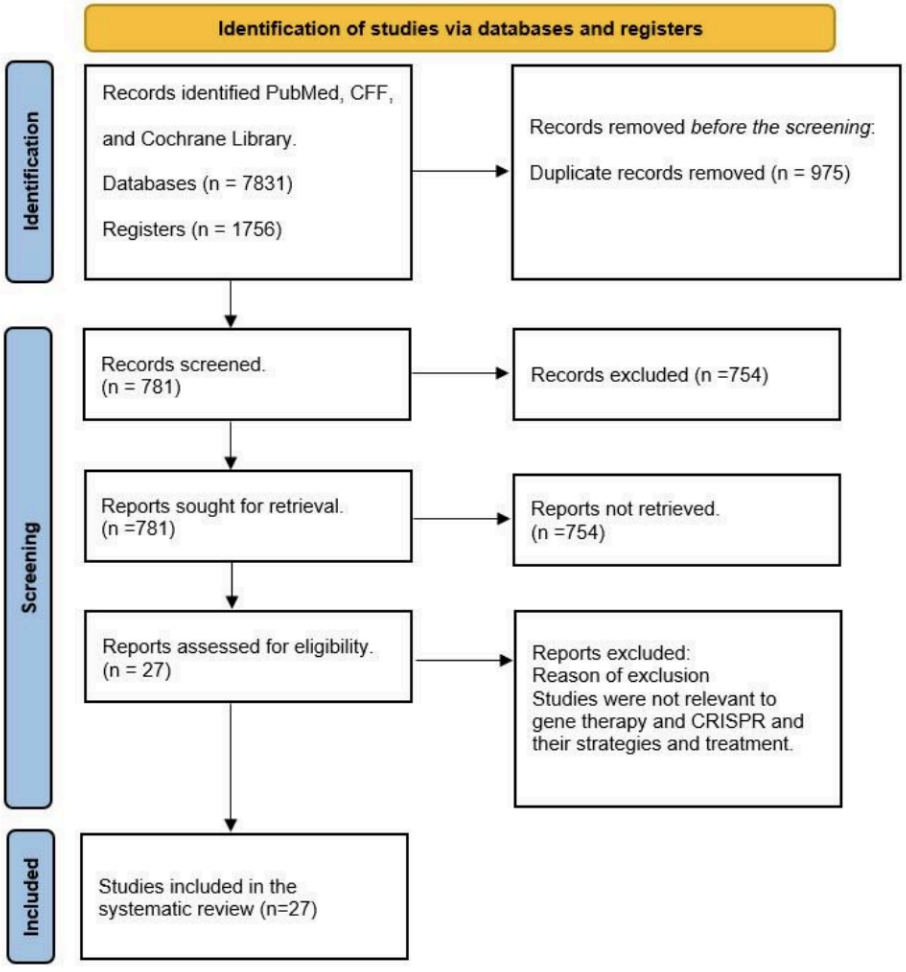

FIGURE 1. PRISMA flowchart of the Systematic Review.

Analysis and Interpretation of Study Findings

Out of the 27 research studies reported, 3 are longitudinal studies (Yang et al., 2021; Stettler et al., 2006; Miller et al., 1982), and the remaining are

cross-sectional studies. A healthy control group was present in all of the studies. The combined number of individuals with CF and controls was 1,839 and 2,178, respectively. The disease is usually diagnosed in early childhood, but some individuals may not be diagnosed until adulthood. The severity of symptoms can vary widely, and individuals with cystic fibrosis may experience a range of complications throughout their lives (Dhooghe et al., 2016).

The majority of the studies were carried out in adults, except those reported by Alton et al. (2015), which included the age group between 10 and 20 years (see Table 1). The findings of the study had different aims, including:

(a) the evaluation and comparison of different techniques for gene therapy and CRISPR (Li et al., 2018) and the change in the nutritional status of individuals with CF (Solomon et al., 2015; Carneiro et al., 2023; Ran et al., 2015); and

(b) the evaluation of lung function and nutritional status for treatment (Hauschild et al., 2016; Lucidi et al., 2009; Alvarez et al., 2016), including the reduction of swelling and inflammation (Boguszewski et al., 2007; Bai et al., 2015), the stage of the disease (Salamoni et al., 1996; Bai et al., 2015), and clinical parameters (Boguszewski et al., 2007).

The Role of Animal Models in Advancing Gene Therapy for Genetic Diseases

CF animal models provide valuable insights into the underlying causes of the disease and have changed the way we think about CF gene therapy. Various animal models have been developed, including pigs, ferrets, rats, zebrafish, and sheep, each with their own advantages and limitations. The advancements in animal models have led to significant breakthroughs in understanding CF pathogenesis and developing new gene therapies. The CF pig model, in particular, has facilitated the measurement of CFTR correction in vivo, which has helped to define the relationship between CFTR expression and Cl^- and $HCO3^-$ transport, with important implications for CF gene therapies (Cooney et al., 2018).

The Promises and Challenges of Gene Editing in Advancing Precision Medicine

Gene editing technologies, such as CRISPR-Cas9, have emerged as promising approaches to modifying nucleic acid sequences in CF research. These tools hold the potential to repair the endogenous CFTR gene and restore its function, but face challenges associated with efficient in vivo

gene delivery. Both gene addition and gene repair strategies rely on efficient in vivo gene delivery to respiratory epithelia and systemic delivery strategies that could correct CF defects in multiple organs. CFTR gene editing has been evaluated in vitro using ZFNs, TALENs, and CRISPR/Cas9 methods (Cooney et al., 2018b). The success of gene therapy, whether for gene addition or gene repair, relies on the vector used to deliver the therapeutic genes. Vectors are utilized as vehicles to introduce the desired genetic material into host cells and can be broken down into viral and non-viral vectors. Viral vectors, which rely on modified viruses to deliver the material, are highly efficient at targeted delivery. Despite this advantage, the use of viral vectors is limited due to restricted gene loading capacity and potential to trigger an immune response, giving rise to safety concerns. Non-viral vectors transport DNA or RNA using either biologically derived or synthetic delivery systems. In comparison to viral vectors, non-viral vectors are able to deliver larger genes and carry a significantly lower risk of an immune reaction (Wang et al., 2023). While non-integrating vectors like adeno-associated virus, adenovirus, and helper-dependent adenovirus are commonly used, a hybrid nonviral transposon/viral integrating vector system has been developed to confer persistent gene expression in mice. The piggyBac transposon has also been shown to promote persistent gene transfer in mice, and a recent study in pigs demonstrated whole lung distribution and phenotypic correction using a piggyBac/Ad vector. HDAd has also shown promise in efficiently transducing airway epithelia, and an integrating piggyBac/HDAd vector could provide a long-term and efficient correction (Limberis & Wilson, 2006).

Assessment of Body Composition Changes as a Clinical Endpoint in Gene Therapy and CRISPR Clinical Trials

The use of body composition as an endpoint can provide valuable information on the effects of gene therapy or CRISPR on the overall health of CF patients. For example, the Alton et al. study discussed earlier assessed body composition changes as one of the secondary endpoints, showing improvements in lean body mass and fat-free mass in patients who received nebulized non-viral CFTR gene therapy. The Banerjee et al. review also highlighted the importance of addressing body composition changes in CF treatment, as current therapies such as CFTR modulators, primarily target the respiratory system by either improving protein folding or enhancing its function. However, these modulators may not fully address the systemic effects of CF, specifically the impacts on the

musculoskeletal and digestive systems. Gene therapy and CRISPR, on the other hand, have the potential to provide more comprehensive and long-lasting treatments for CF, and assessment of body composition changes can aid in evaluating their efficacy (Cooney et al., 2018b).

Delivery Strategies for CRISPR-Cas9 Gene Editing

The potential of CRISPR-Cas9 gene editing technology to revolutionize the treatment of lung diseases caused by genetic mutations is discussed in the reported article (Carneiro et al., 2023). The versatility of CRISPR-Cas9 in terms of application, therapeutic functions, and delivery forms and strategies is highlighted. The article focuses on the use of lipid nanoparticles (LNPs) for efficient encapsulation and protection of CRISPR-Cas9 forms to enhance genome editing. The authors suggest that engineering LNPs as NEMs (nano-engineered microstructures) and administering them as a dry powder through local administration can overcome the pharmacokinetic limitations of systemic administration and ensure persistent accumulation of LNPs loading CRISPR-Cas9 in the lungs (Kazemian et al., 2022). The potential economic advantages of spray-drying technology to produce LNPs loading CRISPR-Cas9 are also discussed. The article concludes that LNPs loading CRISPR-Cas9, administered as a dry powder, could represent the future of lung disease treatment (Yang et al., 2022).

Discussion

This systematic review briefly explains the up-to-date information on gene therapy and CRISPR used in cystic fibrosis. A total of 27 studies were considered appropriate for the current review article based on the various information on gene therapy and CRISPR techniques for the treatment of cystic fibrosis. The research reported on CF is rapidly emerging with the introduction of novel clinical trials, gene therapy, and CFTR modulator therapies. Kosanam et al. (2021) reported the various challenges faced by researchers such as continued safety and efficacy testing of gene therapies, improving accessibility to expensive gene-editing treatments, and enhancement drug therapies that alleviate various symptoms. Although CFTR modulators have demonstrated a high level of success in treating cystic fibrosis, CRISPR technology is the most promising approach to target the root cause of the disease. Ongoing research and testing of CRISPR technology are yielding encouraging results as it offers a cost-effective, efficient, and precise approach to gene editing. This

represents a significant improvement compared to previous genetic engineering tools that were limited in their capabilities. Lino et al. (2018) reported several key aspects, such as the need for personalized treatment strategies, the significance of optimizing drug targeting and delivery, and the potential of using nanotechnology and imaging techniques for improved drug delivery. The authors note that ongoing research efforts in this field are yielding promising results and are likely to lead to the development of more effective treatment options for individuals with cystic fibrosis. The article concludes by emphasizing the importance of continued research and collaboration among scientists, clinicians, and industry partners to advance drug delivery systems and ultimately improve patient outcomes.

Alton et al. (2015) conducted a randomized controlled trial to investigate the safety and efficacy of repeated nebulization of non-viral CFTR gene therapy in patients with cystic fibrosis. The study found that the gene therapy was well-tolerated, but there was no significant improvement in lung function. Bedwell et al. (2017) investigated in vivo genome editing using Staphylococcus aureus Cas9. The study found that the approach was effective in correcting genetic mutations in vivo. Billingsley et al. (2020) investigated in vivo delivery of a CRISPR/Cas9 therapeutic to the deep lung for the treatment of cystic fibrosis. The study found that the approach was effective in editing genes in the lungs of mice. Alton et al. (2021) conducted a phase I/IIa clinical trial to evaluate the safety, tolerability, and pharmacokinetics of aerosolized liposomal VX-661/IVACAFTOR in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. The study found that the drug was well-tolerated, and there was a statistically significant improvement in lung function. Bartlett et al. (2021) investigated systemic and respiratory delivery of CRISPR-Cas9 gene editing using inhaled lipid nanoparticles. The study found that the approach was effective in editing genes in the lungs of mice. Antunes et al. (2018) investigated nanoparticle-mediated delivery of CRISPR-Cas9 genome-editing tools for the treatment of human genetic diseases. The study found that the approach was effective in correcting genetic mutations in vitro and in vivo. The present review has some limitations, including the absence of a formal assessment of the risk of bias in the studies including the involvement of only one reviewer, and a restricted search limited to the PubMed database. Additionally, the outcomes of interest were limited to anthropometric measures.

Conclusion

The studies aimed to evaluate different aspects of cystic fibrosis (CF), including gene therapy, CRISPR, lung function, nutrition status, clinical parameters, and animal models. CF animal models have been valuable in advancing gene therapy for genetic diseases, and gene editing technologies such as CRISPR/Cas9 hold promise in modifying nucleic acid sequences in CF research. Body composition changes have been assessed as a clinical endpoint in CF gene therapy and CRISPR clinical trials and have provided valuable information on the overall health effects of the treatment. Overall, the review provides insights into the current state of research on CF and highlights areas where further research is needed.

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